CYTOGENETICAL STUDIES ON THE GENUS *ORYZA*

2. CHROMOSOME PAIRING IN THE INTERSPECIFIC HYBRID WITH THE ABC GENOMES¹⁾

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Cytological observations by different workers on F_1 hybrids of the cross, *O. sativa* ($2n=24$) $\times$ *O. minuta* ($2n=48$), gave contradictory interpretations on the homology between the A and BC genomes. Okura (1937) and Nandi (1938) considered that one of the genomes of *O. minuta* was homologous to the A genome of *O. sativa*. Kihara and Nezu (1960), Kihara *et al.* (1961) and Nezu *et al.* (1960) reported partial homology between the A and B genomes, whereas Morinaga (1941, 1964), and Morinaga and Kuriyama (1960) concluded that the two genomes were not homologous. On the other hand, from pachytene analysis in the F_1 hybrid of *O. sativa* $\times$ *O. officinalis* ($2n=24$), Shastri *et al.* (1960, 1961) and Katayama (1964, 1965) postulated that the A genome of *O. sativa* is homologous or partially homologous to the C genome of *O. officinalis*.

From cytological standpoint, it is interesting and important to study the homology between the A and BC genomes in the F_1 hybrid of *O. sativa* $\times$ *O. minuta*. In this paper the results on pachytene analysis and chromosome pairing at diakinesis and metaphase I in the F_1 hybrids are reported in relation to the homology between the A and BC genomes.

MATERIALS AND METHODS

Twenty-five hybrids between forms possessing the A genome (*O. sativa*, *O. sativa* f. *spontanea*, *O. breviligulata*, *O. glaberrima*, *O. perennis* and *O. formosana*) and those with the BC genomes (*O. minuta* and *O. punctata*) were used as materials (Table 1). Five F_1 hybrids and 15 species and strains of wild rice were kindly furnished by the National Institute of Genetics at Mishima and the National Institute of Agricultural Sciences at Hiratsuka. Two tetraploid strains (WOO15 and WOO43) used as parents in this experiment and previously labelled as *O. eichingeri* were reidentified as *O. punctata* by Tateoka (1965).

Emasculation was made by hot-water treatment (43°C for 7 minutes). Young seeds at the age of 10-15 days after pollination were sterilized and sown on the artificial

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culture medium in test tube. The cultured seedlings were then transplanted into pots and grown under controlled temperatures in a phytotron. In spring they were moved from the phytotron to natural condition. And later, in order to promote early flower initiation, the plants were subjected to short-day conditions (about 9 hours of daylength) from May until booting.

Anthers were fixed in acetic alcohol (3 : 1) as mentioned earlier (Katayama 1965). Preparations were made by the usual acetocarmine squash method.

RESULTS

In 1963, artificial hybridizations of 24 cross-combinations with the diploid form as the maternal parent were made (Table 1). The total number of flowers pollinated was 778, and 233 crossed seeds were obtained. Almost all of them (93.56%) produced imperfect caryopsis which developed to various degrees. One hundred grains chosen at random from 20 cross-combinations were sown on a culture medium in test tube. Of 61 seedlings which grew up to adult plants, 46 were ascertained as true F_1 hybrids. The remaining 15 plants were, therefore, not observed cytologically. Observations on meiotic pairing of chromosomes in 16 F_1 hybrids were made. All of the F_1 hybrids used in this study were completely sterile.

Table 1. List of cross-combinations between the A and BC genomes used in this experiment

F ₁ plant no.	Cross-combinations	
	(AA)	(BBCC)
2**	<i>O. sativa</i> (521)	× <i>O. minuta</i> (WOO16)
3**	" (108)	× " (WOO45)
5**	" "	× " (WOO51)
6**	" (563)	× "
9**	" (108)	× <i>O. punctata</i> (WOO43)
51	" (Gendjah Ratji)*	× <i>O. minuta</i> *
60	" "	× " (Y 22)*
62	" "	× <i>O. punctata</i> (WOO15)**
61	" (Marich beti)*	× <i>O. minuta</i> (Y 22)*
55	" (P. T. B. 10)*	× "
59	" (Blue Stick)*	× "
52, 58	<i>O. sativa</i> f. <i>spontanea</i> *	× "
54	" "	× <i>O. minuta</i> *
82	" (Y 15)*	× "
75	" (Y 1)*	× <i>O. punctata</i> (WOO15)**
76	<i>O. breviligulata</i> (Y 8)*	× <i>O. minuta</i> (Y 22)*
49	" "	× <i>O. minuta</i> *
57	" "	× " (Y 22)*
56	" (Af 104)**	× "
77	<i>O. glaberrima</i> (W 416)**	× "
78	<i>O. perennis</i> (W 107)**	× "
79	" "	× <i>O. minuta</i> *
80	" "	× <i>O. punctata</i> (WOO15)**
81	<i>O. formosana</i> (Y 16)*	× <i>O. minuta</i> *

* Material obtained from the National Institute of Agricultural Sciences.

** Material obtained from the National Institute of Genetics.

Figs. 1-4. Various stages of chromosome pairings in the hybrids between the A and BC genomes.

1. Pachytene stage, showing $12_{\text{I}} + 12_{\text{I}}$ (F₁#6). 2. Diakinesis, showing $12_{\text{I}} + 12_{\text{I}}$ (F₁#2). 3. MI, showing 36_{I} (F₁#2). 4. MI, showing $6_{\text{I}} + 24_{\text{I}}$ (F₁#60).

1. Pachytene stage

In order to confirm the results obtained from the F₁ hybrid of *O. sativa* × *O. officinalis*, pachytene stage was studied in detail on some F₁ hybrids between the A and BC genomes. The pairing behavior is summarized in Table 2 (F₁#3, F₁#5, and F₁#49) (F₁#3 means abbreviation for F₁ plant no. 3), and illustrated in Figure 1. The 24 chromosome strands, 12 bivalents and 12 univalents, were clearly seen at this stage, that is, the pairing between the homologous chromosomes in the bivalents was completely normal with no evidence of irregularity, except for asynaptic regions which were observed rarely in some chromosome segments. However, there were some PMCs in which 2 or more chromosomes were not well separated from other chromosomes. Bivalents appeared definitely thicker than univalents.

2. Diakinesis and Metaphase I

At early diakinesis, the number of chiasmata per bivalent chromosome counted on F₁#5 ranged from 1 to 3 (mean=1.23). The number of bivalents per cell observed at diakinesis and metaphase I varied from cross to cross (Table 2, Figs. 2-4). As shown

Table 2. Frequency of chromosome pairings per PMC in F₁ hybrids between A and BC genomes

F ₁ plant no.*	Pachytene 12 _I + 12 _{II}	Bivalents						Trivalents			
		Diakinesis			MI			Diakinesis		MI	
		Range	Mean	Total	Range	Mean	Total	Range	Total	Range	Total
2		0-12	1.66	32	0- 7	1.39	38				
3	13	0-10	2.79	17	0- 7	2.58	19				
5	10	3-12	5.58	12	1-11	5.52	68			0- 1	4
6		0- 8	2.31	45	0- 5		10	0- 1	2		
9					0- 8	2.59	47				
51					0-10	4.35	99				
60		3-10		8	0- 8	3.50	99				
62		0- 8	2.74	62	0- 5	1.89	61				
61					0- 3		8				
55		3- 7		6	0- 8	2.92	26			0- 1	1
52		0- 7		7	0-10	4.52	65				
58					0- 9	4.43	94				
54					1- 9	5.31	53				
49	14	0- 8	4.27	13	0- 6	2.38	37				
57					1-10	5.21	58			0- 1	1
56		0-11	4.08	19	1- 6	3.23	13				

* refer to Table 1 for cross-combinations.

in Table 2, the number of bivalents per cell at diakinesis in the F₁ hybrids which gave 20 or more analyzable PMCs ranged from 0 to 8 (mean=2.31) in the minimum (F₁#6) and from 0 to 12 (mean=1.16) in the maximum (F₁#2); at metaphase I, the ranges were from 0 to 5 (mean=1.89) in the minimum (F₁#62) and from 1 to 11 (mean=5.52) in the maximum (F₁#5), respectively. Univalents were either concentrated on the equatorial plate, randomly distributed about it (Fig. 3) or distributed along the spindle. In general, the bivalents had a tendency to be oriented on the equatorial plate (Fig. 4). Almost all bivalents were loose-dumbbell-shaped with a single chiasma (Fig. 4).

In some PMCs of the hybrids, F₁#5, F₁#6, F₁#55 and F₁#57, one trivalent per cell was seldom counted.

DISCUSSION

Roschevicz (1931) grouped *O. sativa* and *O. minuta* into one section, Section *Sativa* Roschev., while Ghose *et al.* (1956) separated them into different sections, namely, Section *Sativa* Ghose and Section *Officinalis* Ghose (cited from Chang 1964). Morishima and Oka (1960) also assigned them into *Sativa* and *Officinalis* groups.

From the frequency of bivalents formed at diakinesis and metaphase I in the F₁ hybrids of the crosses, *O. sativa* × *O. officinalis* and *O. sativa* × *O. minuta*, the genomes of *O. sativa*, *O. officinalis*, and *O. minuta* were designated as AA (Morinaga 1942), CC (Morinaga and Kuriyama 1959), and BBCC (Morinaga 1943), respectively.

As shown in Table 3, from the F₁ hybrids of the crosses, *O. sativa* × *O. minuta*, *O. sativa* × *O. eichingeri*, *O. minuta* × *O. sativa* and *O. glaberrima* × *O. eichingeri*, Morinaga (1941, 1963) and Morinaga and Kuriyama (1960) observed 36 univalents in the majority

Table 3. Range and mean of chromosome pairings in the F₁ hybrids between A and BC genomes as reported in literature

Authors	Number of		Cross-combinations
	bivalents	trivalents	
Okura (1937)	10-12	0-2	<i>O. sativa</i> × <i>O. minuta</i>
Nandi (1938)	12		<i>O. minuta</i> × <i>O. sativa</i>
Morinaga (1941)	0-3		<i>O. sativa</i> × <i>O. minuta</i>
Morinaga & Kuriyama (1960)	0-3		" × "
	0		<i>O. minuta</i> × <i>O. sativa</i>
	0		<i>O. sativa</i> × <i>O. eichingeri</i>
Kihara & Nezu (1960)	0-9(6.0)*		" × <i>O. minuta</i>
Nezu <i>et al.</i> (1960)	0-9(4.5)*	0.1	" (<i>indica</i>) × "
	1-8(4.7)*		" (<i>japonica</i>) × "
	0-9(4.0)*		<i>O. breviligulata</i> × <i>O. eichingeri</i>
Li <i>et al.</i> (1962)	1-5(3.0)*	0	<i>O. sativa</i> × <i>O. minuta</i>
	0-2(0.14)*	6	" (<i>spontanea</i>) × <i>O. eichingeri</i>
	very low		<i>O. sativa</i> × <i>O. schweinfurthiana</i>
Bouharmont (1962)	very rare		" (4n) × "
Morinaga (1964)	0		<i>O. glaberrima</i> × <i>O. eichingeri</i>

()* indicates mean of chromosome pairings.

of PMCs, with a few cases of bivalent formation not more than three pairs. Li *et al.* (1962) also obtained similar results in the hybrids of the crosses, *O. sativa* × *O. minuta* and *O. sativa* f. *spontanea* × *O. eichingeri*. The mean numbers of bivalents per cell at metaphase I were 3.0 (range 1-5) for the former and 0.14 (range 0-2) for the latter.

Okura (1937) and Nandi (1938), on the other hand, reported the formation of 12 bivalents in the F₁ hybrids between *O. sativa* and *O. minuta* (Table 3). Okura concluded that one of the genomes of *O. minuta* was homologous to the genome of *O. sativa*. Nandi concluded that *O. minuta* was an allo-octoploid species which had probably originated by hybridization between *O. officinalis* and *O. sativa* (1936), and proposed that the B genome of *O. minuta* is homologous to the A genome of *O. sativa* (1938).

From the mean number of bivalents in the F₁ hybrids of *O. sativa* × *O. minuta* and *O. breviligulata* × *O. eichingeri*, Kihara and Nezu (1960), Kihara *et al.* (1961) and Nezu *et al.* (1960) considered that the B genome of *O. minuta* and *O. eichingeri* was partially homologous to the A genome of *O. sativa* and *O. breviligulata* (Table 3).

From the morphological and geographical standpoints, Sampath proposed that there is a close relationship among the B, C, and D genomes (unpubl.).

In the F₁ hybrids used in the present experiment, the author could observe 12 bivalents and 12 univalents at pachytene stage, and as many as 12 bivalents (mean = 5.58) per cell at diakinesis (F₁#5) and 11 (mean = 5.52) at metaphase I (F₁#5). This fact shows that there is partial homology between the genome A and one of the BC genomes.

From these descriptions, the bevalents formed in the F₁ hybrids between the A and BC genomes were roughly divided into four groups as follow: 1) intergenomic pairing of the chromosomes between A and C, 2) intergenomic pairing of the chromosomes between A and B, 3) intergenomic pairing of the chromosomes between B and C, and 4) intragenomic pairing of the chromosomes.

At pachytene stage in the F_1 hybrid of the cross, *O. sativa* $\times$ *O. officinalis*, Shastry *et al.* (1960, 1961) observed complete pairing between the chromosome complements of the constituent species, concluding that the A genome was homologous to the C genome. The author also observed that the pairing behavior in the F_1 hybrids of *O. sativa* (563) $\times$ *O. officinalis* (WOOO6) and *O. sativa* (Norin No. 8) $\times$ *O. officinalis* was similar to that reported by Shastry *et al.* (1960, 1961), and concluded that the A genome was partially homologous to the C genome (Katayama 1965).

From the morphological features, on the other hand, McClintock (1933) described a non-homologous association of parts of chromosomes at pachytene in *Zea*. Shastry and Ranga Rao (1961), Li *et al.* (1963) and Wu *et al.* (1963) reported that almost all bivalents or some of them observed at metaphase I in the F_1 hybrids of *O. sativa* $\times$ *O. australiensis* and *O. minuta* $\times$ *O. brachyantha* were autosyndetic.

In the present experiment, no evidence of non-homologous association was observed at pachytene stage. There was no distinguishable differences of chromosome size in both *O. sativa* and *O. minuta*. Some of bivalents and trivalents observed in those hybrids might be autosyndetic, but the author assumed that majority of bivalents come from allosyndetic pairing, because chiasma formation was observed between homologous chromosomes at early diakinesis stage.

On the basis of pachytene analysis and the range of bivalents per cell at diakinesis and/or metaphase I in the F_1 hybrids of the crosses, *O. sativa* $\times$ *O. officinalis* (Shastry *et al.* 1960, 1961; Katayama 1965), *O. sativa* $\times$ *O. minuta*, and *O. sativa* $\times$ *O. punctata*, the author concludes that the C genome of *O. minuta* and *O. punctata* is partially homologous to the A genome of *O. sativa*. Their relationship will be made more clear in future publications dealing with the F_1 hybrids between the A and CD genomes and with two backcrossed hybrids, (A $\times$ BC) $\times$ A and (A $\times$ CD) $\times$ A.

The trivalents which were seldom formed in the F_1 hybrids, *O. sativa* $\times$ *O. minuta* and *O. sativa* $\times$ *O. punctata*, may arise from the pairing of the chromosomes among the A, B, and C genomes.

SUMMARY

Twenty-five hybrids between forms possessing the A genome (*O. sativa*, *O. sativa* f. *spontanea*, *O. breviligulata*, *O. glaberrima*, *O. perennis* and *O. formosana*) and those with the BC genomes (*O. minuta* and *O. punctata*) were used as materials.

From 778 flowers pollinated, 233 seeds (almost all of them were imperfect) of various degrees of development were produced. One hundred grains chosen at random from 20 cross-combinations were sown on the culture medium in test tube. Sixteen true F_1 plants were studied cytologically.

At pachytene stage, 12 bivalents and 12 univalents were observed. At diakinesis and metaphase I, the number of bivalents ranged from 3 to 12 or from 1 to 11, respectively.

From the previous results of pachytene analysis in the F_1 hybrid of the cross, *O. sativa* $\times$ *O. officinalis* (Shastri *et al.* 1960, 1961; Katayama 1965), and of pachytene analysis and of bivalent formation at diakinesis and at metaphase I in the F_1 hybrids, *O. sativa* $\times$ *O. minuta* and *O. sativa* $\times$ *O. punctata*, the author could conclude that the C genome of *O. minuta* and *O. punctata* is partially homologous to the A genome of *O. sativa*.

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